pyridines. Briefly, NADPH-fortified microsomes (usually 50 pmol of cytochrome P-450 equivalent) were incubated with 0.2 mM dihydropyridine in 100 mM potassium phosphate buffer (pH 7.85) at 37 °C for 10 min (total volume 0.5 mL). A 100-μL portion of 1 M Na_2CO_3 buffer (pH 10.5) containing 2 M NaCl was added, and the solution was extracted twice with 1.0 mL of CH_2Cl_2. Measured portions of the combined CH_2Cl_2 extracts were withdrawn each time and dried under N_2. Residues were dissolved in CH_3OH, and aliquots were assayed by HPLC. Either a 6.2 mm × 8 cm 3 μm DuPont (DuPont Instruments, Wilmington, DE) Zorbax Gold octadecylsilyl (C_{18}) column (1–5, 8, 9, 13–19) or a 4.6 mm × 25 cm 5 μm DuPont Zorbax octylsilyl (C_8) column (6, 7, 10, 12) was used with a Spectra-Physics 8700 system (Spectra-Physics, Piscataway, NJ) and Laboratory Data Control UV II (Rivera Beach, FL) monitor. The solvent systems used for particular compounds included CH_3OH and H_2O, and all separations were performed isocratically. HPLC method 1: C_{18}, CH_3OH/H_2O 60:40 (v/v), flow 2.5 mL min^{-1}. HPLC method 2: C_8, CH_3OH/H_2O 60:40 (v/v), flow 1.3 mL min^{-1}. HPLC method 3: C_{18}, CH_3OH/H_2O 60:40 (v/v), flow 2.0 mL min^{-1}. HPLC method 4: C_{18}, CH_3OH/H_2O 75:25 (v/v), flow 2.0 mL min^{-1}. The detection wavelength was 254 nm for the assays with compounds 1, 6–14, and 16–19 and both 254 and 280 nm (two detectors in line) for the assays with compounds 2–5. Quantitative measurements were generally made with external standards and measurements based upon peak heights.

Other Assays. Cytochrome P-450 measurements were made as described.[43] Phenacetin *O*-deethylase activity was measured by TLC as described elsewhere.[44] Hexobarbital 3′-hydroxylase activity was estimated by HPLC as described by Rummelt et al.[45]

Acknowledgment. This work was supported in part by USPHS Grants CA 30907 and ES 00267 and the Deutsche Forschungsgemeinschaft (Boe 784/1-1).

Registry No. **1a**, 21829-25-4; **2a**, 103026-73-9; **3a**, 103026-74-0; **4a**, 103026-75-1; **5a**, 17438-14-1; **5b**, 27525-74-2; **6a**, 51384-20-4; **6b**, 103026-76-2; **7a**, 32947-20-9; **7b**, 103026-77-3; **8a**, 73257-48-4; **8b**, 103026-78-4; **9a**, 86408-11-9; **9b**, 103026-79-5; **10a**, 103026-84-2; **10b**, 103026-80-8; **11a**, 103026-85-3; **11b**, 103026-81-9; **12a**, 73257-45-1; **12b**, 103026-82-0; **13a**, 1165-06-6; **13b**, 1539-44-2; **14a**, 70677-78-0; **14b**, 77234-00-5; **15a**, 1153-66-8; **16a**, 63675-72-9; **16b**, 103026-83-1; **17a**, 66085-59-4; **17b**, 85677-93-6; **18a**, 22609-73-0; **18b**, 88443-04-3; **19a**, 39562-70-4; **19b**, 89267-41-4; **20a**, 1149-23-1; **20b**, 1149-24-2; $PhCH_2CHO$, 122-78-1; $(CH_3)_2CHCHO$, 78-84-2; CH_2O, 50-00-0; C_6H_5CHO, 100-52-7; C_2H_5CHO, 123-38-6; $CH_3COCH_2CH_2CH_3$, 105-45-3; $CH_3COCH_2CO_2CH_2CH_3$, 141-97-9; 3-cyclohexene-1-carboxaldehyde, 100-50-5.

(43) Omura, T.; Sato, R. *J. Biol. Chem.* **1964**, *239*, 2370.
(44) Larrey, D.; Distlerath, L. M.; Dannan, G. A.; Wilkinson, G. R.; Guengerich, F. P. *Biochemistry* **1984**, *23*, 2787.
(45) Rummelt, V.; Ott, R.; Böcker, R. *Drugs Exp. Clin. Res.*, in press.

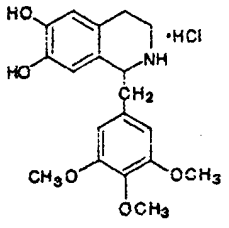
Syntheses and β-Adrenergic Agonist and Antiaggregatory Properties of N-Substituted Trimetoquinol Analogues[1]

Adeboye Adejare,[†] Duane D. Miller,*[†] Joanne S. Fedyna,[‡] Chang-Ho Ahn,[‡] and Dennis R. Feller[‡]

Divisions of Medicinal Chemistry and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.
Received September 16, 1985

Trimetoquinol [1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, TMQ] is a potent β-adrenergic receptor agonist and inhibitor of human platelet aggregation. Selective cleavage of *O*-benzyl groups in the presence of an *N*-benzyl group using HCl and formation of a cyclic sulfite ester from the reaction of a catechol with thionyl chloride were achieved. The N-substituents included methyl, benzyl, and β-hydroxy- and β-chloroethyl groups. Each N-substituted TMQ caused a concentration-dependent stimulation of β_2 (trachea) and β_1 (atria) adrenoceptor tissues and inhibition of 15(*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5,13(*E*)-dienoic acid (U46619, a thromboxane A_2 mimetic) mediated human platelet activation. TMQ remained the most potent in the series. Structure–activity results indicated that the larger the N-substituent, the lower the β-adrenergic activity but the higher the inhibition of platelet aggregatory activity. Thus, the structural requirements of these TMQ analogues for the two types of biological activity are different.

Tetrahydroisoquinolines (THIs) represent a class of cyclized phenethylamines whose pharmacological properties include lipolytic,[2,3] bronchial relaxant,[4–7] hypotensive,[8,9] platelet antiaggregatory,[10] cardiostimulant,[4–7,11] and uterine-stimulant[5] properties. Structural requirements for potent bronchodilating activity in the THI series have been proposed to include the 1,2,3,4-THI nucleus, the catechol hydroxy groups at positions 6 and 7, and an arylmethyl group at position 1.[6,12] 1-(3,4,5-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (trimetoquinol, TMQ; **1**) is one of the most potent β-adrenoceptor stimulants examined thus far and is approximately 10 times more active than the classical β-adrenoceptor agonist isoproterenol in guinea pig tracheal relaxation.[11] Potent β-adrenoceptor activation residues primarily with the *S*-(−) isomer of TMQ (Inolin) and is currently used in Japan for relief of asthmatic bronchospasms.[11]



1

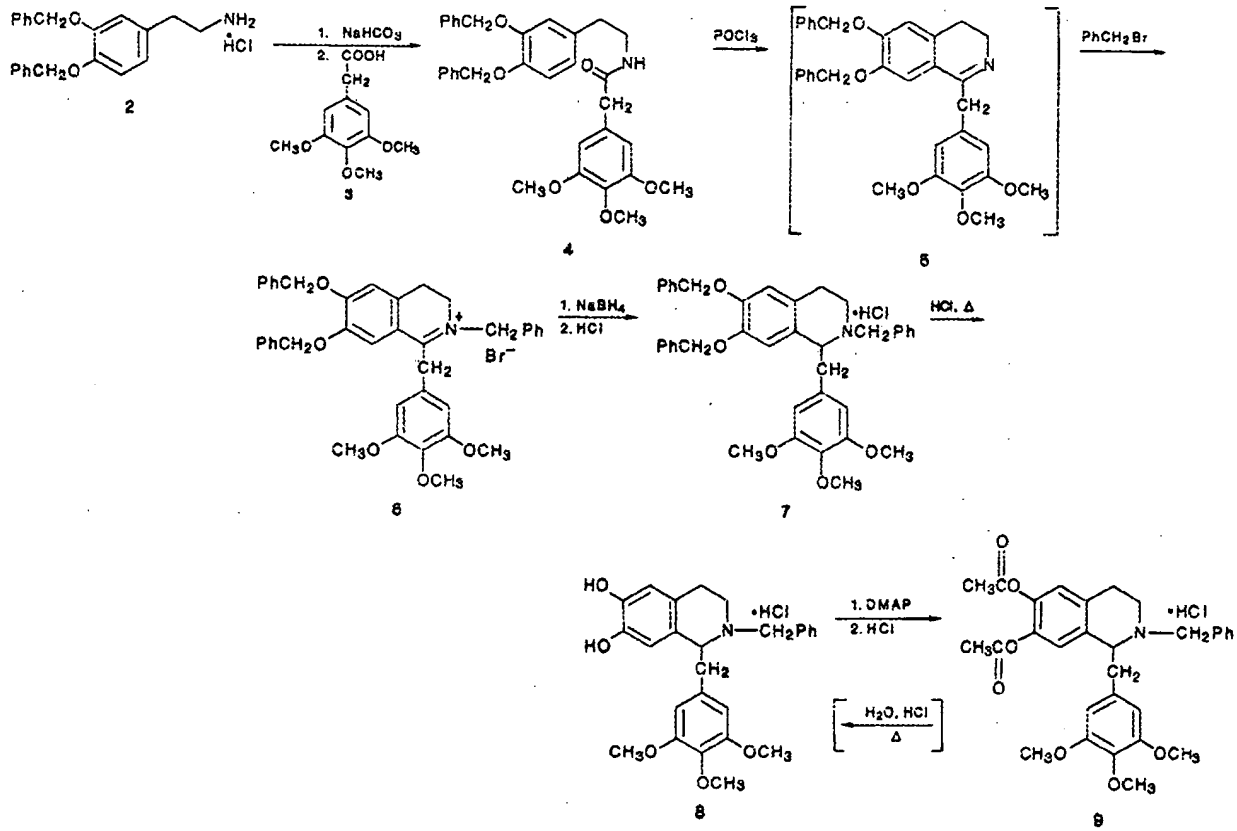
Trimetoquinol and related analogues[13] also have been found to be potent inhibitors of platelet aggregation. The

[†] Division of Medicinal Chemistry.
[‡] Division of Pharmacology.

(1) (a) Taken in part from the Ph.D Dissertation of A. Adejare, The Ohio State University, 1985. (b) Adejare, A.; Ahn, C.; Fedyna, J. S.; Feller, D. R.; Miller, D. D. Presented at the 189th National Meeting of the American Chemical Society, Miami, FL, April 1985.
(2) Shonk, R. F.; Miller, D. D.; Feller, D. R. *Biochem. Pharmacol.* **1971**, *20*, 3403.

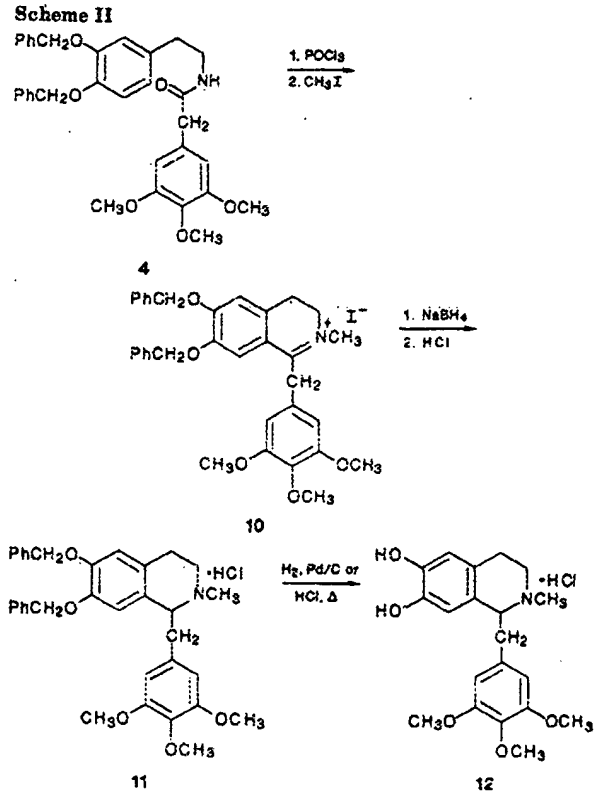
0022-2623/86/1829-1603$01.50/0

Scheme I



antiaggregatory activity of TMQ is independent of β-adrenergic agonist activity.[10] Both isomers of TMQ (*R*-(+) and *S*-(−)) have been shown to inhibit the prostaglandin-dependent pathway of human platelet aggregation. However, (*R*)-(+)-TMQ is at least 10-fold more potent than (*S*)-(−)-TMQ as an inhibitor of aggregation induced by collagen, ADP (secondary phase), arachidonic acid, cyclic endoperoxide analogues (U46619 and U44069), and thromboxane A_2 (TXA_2). More recent work from our laboratories has indicated that (*R*)-(+)-TMQ may act by antagonizing TXA_2 at its receptor.[13,14] Only a few TMQ analogues have been evaluated for antiplatelet activity thus far, and all have shown a lower activity in inhibiting platelet aggregation than stimulating β-adrenoceptors.

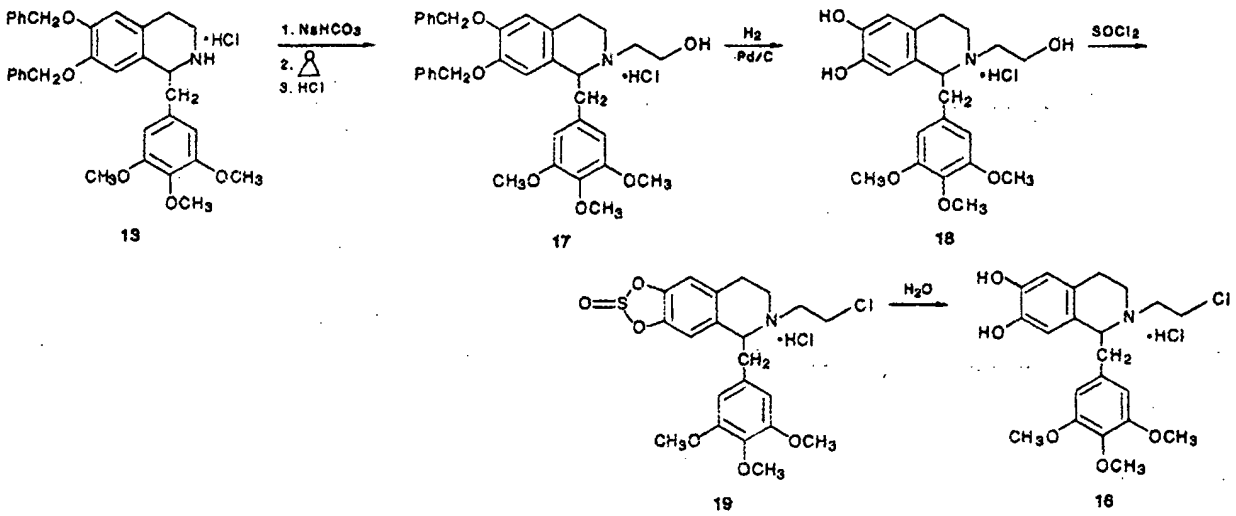
Scheme II



The preparation and biological evaluation of a series of N-substituted TMQ analogues has not been done. Synthesis of *N*-methyl-TMQ has not been reported though it

(3) Lee, O. S.; Mears, J. A.; Miller, D. D.; Feller, D. R. *Eur. J. Pharmacol.* 1974, *28*, 225.
(4) Buckner, C. K.; Abel, P. *J. Pharmacol. Exp. Ther.* 1974, *189*, 616.
(5) Brittain, R. T.; Jack, D.; Ritchie, A. C. *Adv. Drug Res.* 1970, *5*, 197.
(6) Yamato, E.; Hirakura, M.; Sugasawa, S. *Tetrahedron, Suppl.* 1966, *8*(*Part 1*), 129.
(7) Feller, D. R.; Venkatraman, R.; Miller, D. D. *Biochem. Pharmacol.* 1975, *24*, 1357.
(8) Laidlaw, P. P. *J. Physiol. (London)* 1910, *40*, 480.
(9) Privitara, P. J.; Blickenstaff, T.; Gaffney, T. E.; Mohammed, S. *J. Pharmacol. Exp. Ther.* 1971, *176*, 656.
(10) Shtacher, G.; Crowley, H. J.; Dalton, C. *Biochem. Pharmacol.* 1976, *25*, 1045.
(11) Iwasawa, Y.; Kiyomoto, A. *Jpn. J. Pharmacol.* 1967, *17*, 143.
(12) Holtz, P. *Pharmacol. Rev.* 1966, *18*, 85.
(13) Mayo, J. R.; Navran, S. S.; Huzoor-Akbar; Miller, D. D.; Feller, D. R. *Biochem. Pharmacol.* 1981, *30*, 2237.
(14) Mukhopadhyay, A.; Navran, S. S.; Amin, H. M.; Abdel-Aziz, S. A.; Chang, J.; Sober, D. J.; Miller, D. D.; Feller, D. R. *J. Pharmacol. Exp. Ther.* 1985, *232*, 1.

Scheme III



13 17 18 19 16

has been shown to possess weak β-adrenoceptor activity[14] as compared to TMQ. However, this N-substituted analogue was only 5-fold less active as an antagonist of TXA_2/endoperoxide-mediated platelet activation. The objective of this investigation was to synthesize several N-substituted TMQ derivatives. These drugs will be evaluated for their potency and selectivity in β-adrenergic receptor systems and for their ability to inhibit platelet aggregation. Compounds 8, 16, 18, and 12 have been proposed as suitable targets for achieving this goal. Additionally, the potential alkylating agent 16 has been designed to determine whether the β-adrenoceptor and TXA_2 blocking properties of TMQ are altered in an irreversible fashion.

Chemistry. Syntheses of the desired TMQ derivatives were achieved as shown in Schemes I–III. Condensation of the free base of phenethylamine 2 with the acetic acid derivative 3 gave the amide 4 in 75% yield and forms an improvement over the previously reported procedure.[15] Bischler–Napieralski cyclization of amide 4 gave the unstable imine 5, a key intermediate. This imine can be reacted with alkyl halides such as benzyl bromide and methyl iodide to give the iminium salt. The iminium salts can then be reduced with sodium borohydride and HCl salts isolated.

Several attempts to selectively cleave the *O*-benzyl groups in the presence of the *N*-benzyl group were made. Some of the systems tried were hydrogenation with Pd/C or $Pd/BaSO_4$[16,17] as catalyst, Me_3SiI,[18,19] and BCl_3.[19] Use of 10% Pd/C at 45 psi for 16 h or 5% $Pd/BaSO_4$ at atmospheric pressure for 10 h gave TMQ. Attempts to monitor the hydrogenation using $Pd/BaSO_4$ as catalyst as well as the Me_3SiI and BCl_3 reactions gave complex mixtures, and this work was not pursued. The mixtures probably correspond to the several debenzylation combinations possible and perhaps demethylations in the latter two cases.

After trying several HCl–solvent systems and conditions, a best system, which involved refluxing amine salt 7 in a 50:50 mixture of concentrated HCl and methanol, was discovered. Reflux for 32 h gave the desired *N*-benzyl-TMQ. This molecule always crystallized with the solvent used for crystallization, which was either ethanol or tetrahydrofuran. Although further search in the literature revealed precedence for cleaving an *O*-benzyl group to give a phenol by this method,[20] this may be the first report of obtaining a catechol in this manner and the type of selectivity described by use of this system.

Confirmation that the desired catechol 8 was obtained was achieved by acetylating[21,22] the catechol product, characterizing the diacetyl derivative 9 and reconverting it to catechol 8 by simple ester hydrolysis. The products obtained by the two methods were shown to be identical. The *N*-methyl-TMQ analogue was obtained from amine salt 11 by hydrogenation and HCl debenzylation, with the latter procedure giving a slightly higher yield.

Amine salt 13 reacted with chloroacetic anhydride in the presence of Na_2CO_3 to give amide 14. Amide 14 could not be converted to the desired catecholamine 16 because it could not be reduced to give isolatable β-chloroethyl amine 15 by diborane,[23-25] triethylsilane,[23] and trichlorosilane.[25] Reaction of the free base of amine salt 13 with ethylene oxide while cooling with ice–water produced the desired amine alcohol 17. The amine alcohol salt could not be chlorinated to give isolatable β-chloroethyl amine salt 15 by use of thionyl chloride,[26,27] phosphorus oxychloride,[28] triphenylphosphine/carbon tetrachloride,[29] or methane-

(15) Miller, D. D.; Osei-Gyimah, P.; Bardin, J.; Feller, D. R. *J. Med. Chem.* **1975**, *18*, 454.
(16) Freifelder, M. *Catalytic Hydrogenation in Organic Synthesis: Procedures and Commentary*; Wiley: New York, 1978; p 119.
(17) Kirby, G. W.; Tiwari, H. P. *J. Chem. Soc. C* **1966**, 676.
(18) Cheng, H.; Doskotch, R. W. *J. Nat. Prod.* **1980**, *43*, 153.
(19) Olah, G. A.; Narang, S. C. Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247.
(20) Menger, F. M.; Chow, J. F.; Kaiserman, H.; Vasquez, P. C. *J. Am. Chem. Soc.* **1983**, *105*, 4997.
(21) Bindra, J. S.; Grodski, A. *J. Org. Chem.* **1977**, *42*, 910.
(22) Hofle, G.; Steglich, W.; Vorgruggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569.
(23) Kador, P. F.; Venkatraman, R.; Feller, D. R.; Miller, D. D. *J. Med. Chem.* **1977**, *20*, 891.
(24) Granchelli, F. E.; Soloway, A. H.; Neumeyer, J. L.; Filer, C. N. *J. Org. Chem.* **1977**, *42*, 2014.
(25) Ginos, J. Z.; Cotzias, G. C.; Tolosa, E.; Teng, L. C.; LaMonte, A. *J. Med. Chem.* **1975**, *18*, 1199.
(26) Hayashi, T.; Konishi, M.; Fukushima, M.; Konchira, K.; Kioki, T.; Kumada, M. *J. Org. Chem.* **1983**, *48*, 2195.
(27) Kammerer, R. C.; Amiri, B.; Cho, A. K. *J. Med. Chem.* **1979**, *22*, 352.
(28) Omar, A. M. M. E.; Habib, N. S.; Aboulwafa, O. M. *J. Pharm. Sci.* **1982**, *71*, 991.

Table I. β_1- and β_2-Adrenoceptor Activities of Trimetoquinol (TMQ) Isomers and N-Substituted Analogues in Isolated Guinea Pig Atrial and Tracheal Preparations

compound	pD_2[a,b] (mean ± SEM)			
	atrium (β_1)	n	trachea (β_2)	n
(*R*)-(+)-TMQ	5.58 ± 0.17	3	5.63 ± 0.10	4
(*S*)-(−)-TMQ	7.26 ± 0.20	4	7.23 ± 0.16	3
(±)-TMQ (1)	7.10 ± 0.08	5	6.96 ± 0.07	7
TMQ-NCH_3 (12)	5.89 ± 0.10	3	6.05 ± 0.05	6
N-β-hydroxyethyl-TMQ (18)	5.35 ± 0.01	3	5.73 ± 0.06	7
N-β-chloroethyl-TMQ (16)	4.88 ± 0.10	3	5.34 ± 0.01	5
cyclic sulfite *N*-β-chloroethyl-TMQ (19)	5.24 ± 0.14	3	5.58 ± 0.12	5
N-benzyl-TMQ (8)	4.34 ± 0.07	2	5.34 ± 0.10	8

[a] $pD_2 = -\log ED_{50}$ where ED_{50} = concentration required to produce a relaxation equal to 50% of the maximal effect by the drug. [b] TMQ gave maximal responses equal to 98% and 91% of isoproterenol in trachea and atria, respectively. All TMQ analogues gave maximal effects similar to those of TMQ in these two tissues.

sulfonyl chloride/lithium chloride.[30] Each system usually resulted in a complex mixture.

Cleavage of the dibenzyl protecting groups of amine alcohol salt 17 gave the catechol β-hydroxy TMQ 18. Treatment of this with $SOCl_2$ was expected to give the β-chloroethyl TMQ directly.[31] However, another compound, the cyclic sulfite β-chloroethyl TMQ 19 crystallized out in 87% yield during the reaction. This was surprising because a similar reaction in the literature was reported to give only the desired β-chloro adduct without $SOCl_2$ reacting with the catechol hydroxy groups.[31] This is the first report of catechols reacting with $SOCl_2$ and providing a cyclic ester ring system. Use of cyclic esters of this type as substrates for nucleophilic substitution in carbohydrate chemistry was recently described.[32] The cyclic sulfur esters were prepared in a similar manner to the above except for substituting a diol for the catechol. The β-chloroethyl TMQ 16 was then obtained by simple aqueous hydrolysis of the sulfur ester 19 followed by lyophilization to give the desired product in 99% yield.

Biological Results and Discussion

Preliminary pharmacological data obtained on the N-substituted TMQ analogues show that the analogues retained both β-adrenergic and antiplatelet aggregatory effects. The cyclic sulfite intermediate 19 also was evaluated.

β-Adrenergic Studies. The results of preliminary evaluations on carbachol-contracted guinea pig tracheal strips (β_2-adrenoceptor system) and spontaneously beating guinea pig right atria (β_1-adrenoceptor system) are shown in Table I. The rank order of β-adrenoceptor agonist activity of these compounds was (*S*)-(−)-TMQ > (±)-TMQ (1) > 12 > (*R*)-(+)-TMQ ≥ 18 > 19 > 16 ≥ 8. As expected, (*S*)-(−)-TMQ was considerably more potent than (*R*)-(+)-TMQ and more potent than (±)-TMQ in both β-adrenoceptor systems. For the N-substituted analogues (8, 12, 16, and 18), it was found that the bigger the N-substituent, the lower the β-adrenergic agonist activity. However, activation of β_2-adrenoceptors by these analogues was able to tolerate this bulk better, leading to a 10-fold difference in activity of the *N*-benzyl analogue 8 in the β-adrenoceptor systems.

To determine whether compound 16, shown to be a β-agonist (Table I), is capable of interacting irreversibly with β-adrenoceptors, evaluation of binding to β_1 (atrial tissue) adrenoceptors was carried out. The concentration–response curves of other agonists were evaluated in tissues with and without preexposure to analogue 16. Prior incubation (1.5–2 h) of guinea pig atria with 16, followed by a 2-h washout, did not produce a marked change in the concentration–response curves of other analogues. This preliminary data indicates that compound 16 does not give irreversible alkylation of β-adrenoceptors in guinea pig atria.

Table II. Inhibition of U46619-Induced Human Platelet Aggregation and Secretion of [^{14}C]Serotonin by Trimetoquinol (TMQ) Isomers and N-Substituted Analogues

compound[b]	IC_{50},[a] μM	
	aggregation	secretion
R-(+)-TMQ	0.71 ± 0.16	0.55 ± 0.13
S-(−)-TMQ	19.3 ± 2.8	24.5 ± 3.0
N-benzyl TMQ (8)	21.2 ± 0.4	96.8 ± 5.7
N-β-hydroxyethyl-TMQ (18)	71.6 ± 3.6	116.2 ± 6.9
N-β-chloroethyl-TMQ (16)	86.6 ± 15.3	162 ± 28.3
cyclic sulfite *N*-β-chloroethyl-TMQ (19)	64.2 ± 5.8	166.4 ± 31.1

[a] IC_{50} = concentration of each antagonist required to reduce the biological response by 50%. Values represent the mean ± SEM of data from 3–4 donors. [b] Analogue 12 was previously reported.[14] This compound gave an IC_{50} value of 40–50 μM against U46619-induced platelet activation.

Platelet Studies. The antiaggregatory and antisecretory potencies of the N-substituted analogues in washed human platelets are shown in Table II. Each of the compounds blocked the aggregatory and secretory responses to U46619, a TXA_2 agonist[33] in human platelets. With use of (*R*)-(+)-TMQ as a reference antagonist, each of the analogues was less active. The rank order of inhibitory potencies (IC_{50}) for the compounds was, against aggregation, (*R*)-(+)-TMQ > (*S*)-(−)-TMQ > 8 > 19 > 18 > 16, and against secretion, (*R*)-(+)-TMQ > (*S*)-(−)-TMQ > 8 > 18 > 16 = 19. The isomeric activity differences for the TMQ isomers against U46619-induced platelet activation are similar to those of an earlier report.[13]

N-Benzyl-TMQ 8 was found to be the most potent inhibitor of aggregation and serotonin secretion induced by U46619. Although *N*-benzyl-TMQ 8 was about 30-fold less potent than (*R*)-(+)-TMQ, it also was the least potent β-adrenoceptor stimulant among the N-substituted analogues. On the basis of our findings, these N-substituted analogues appear to act like TXA_2 antagonists, as has previously been proposed for the parent drug, TMQ.[13] Additional work with other stimuli is needed to demonstrate the specificity of their effects against U46619 (a TXA_2 mimetic) response in human platelets. If these compounds are TXA_2 antagonists, then *N*-benzyl-TMQ 8 offers some promise for the development of selective an-

(29) Sayre, L. M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* 1983, *26*, 503.
(30) Brueggemeier, R. W.; Snider, C. E.; Counsell, R. E. *Cancer Res.* 1982, *42*, 3334s.
(31) Cross, A. J.; Waddington, J. L.; Ross, S. T. *Life Sci.* 1983, *32*, 2733.
(32) Tewson, T. J. *J. Org. Chem.* 1983, *48*, 3507.
(33) Coleman, R. A.; Humphrey, P. P. A.; Kennedy, I.; Levy, G. P.; Lumley, P. *Br. J. Pharmacol.* 1981, *73*, 773.

tagonists of TXA_2 receptor mediated biological responses.

Unlike the rank order potency found for these TMQ analogues in β-adrenoceptor systems, the TXA_2 receptor system seems to accept a rather bulky alkylaryl N-substituent and does not show a correlation between the TXA_2 blocking activity and increasing size of the substituents. For example, the *N*-methyl analogue (**12**)[14] is more active than each of the remaining N-substituted analogues (**16**, **18**, and **19**). Thus, our plans are to examine the influence of substituents on the aryl segment of the *N*-benzyl group of **8**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained with a Beckman 4230 infrared spectrophotometer, and NMR spectral data were obtained with a Bruker HX-90E (90 MHz) or Bruker WP-80DS (80 MHz) NMR spectrometer. Mass spectra were obtained with a DuPont 21-491 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN. Analytical results for elements indicated were within ±0.4% of the theoretical values. Hexane and ethyl acetate were distilled before use. Acetonitrile was refluxed over P_2O_5 overnight and then distilled while THF was dried by refluxing over sodium overnight, distilling, and then redistilling over sodium and benzophenone (as the deep blue indicator for dryness). Methanol was distilled over magnesium methoxide, generated in situ from magnesium, iodine, and methanol. Ethanol was distilled over magnesium ethoxide generated in a similar manner to that for magnesium methoxide.

***N*-[3,4-Bis(benzyloxy)phenethyl]-3,4,5-trimethoxyphenylacetamide (4).** A solution of 16.70 g (42.4 mmol) of commercially available 3,4-bis(benzyloxy)phenethylamine hydrochloride (**2**) in 100 mL of $CHCl_3$ was extracted three times with 100 mL of saturated $NaHCO_3$ solution and washed with H_2O. The $CHCl_3$ solution was dried over $MgSO_4$, and $CHCl_3$ was removed under vacuum. To the amine residue was added 9.50 g (42.0 mmol) of 3,4,5-trimethoxyphenylacetic acid (**3**) and 100 mL of toluene. The mixture was then refluxed for 3 days with azeotropic removal of water by use of a Dean–Stark trap. It was allowed to cool and the toluene removed under vacuum. The residue was redissolved in 100 mL of $CHCl_3$ and washed three times with 100 mL of 10% HCl, once with 100 mL of H_2O, three times with 100 mL of saturated $NaHCO_3$, and twice with 100 mL of H_2O. The organic layer was then dried over $MgSO_4$ and the $CHCl_3$ removed under vacuum to give the crude amide as a brown solid. The solid was crystallized from hot toluene and washed with ether to give 17.00 g (75%) of a white crysalline material: mp 109–111 °C (lit.[13] mp 108–109 °C); ^1H NMR ($CDCl_3$) δ 7.45–7.28 (m, 10 H, Ar H), 6.85–6.73 (m, 2 H, Ar H), 6.55–6.44 (m, 1 H, Ar H), 6.35 (s, 2 H, Ar H), 5.50–5.35 (br, 1 H, NH), 5.12, 5.11 (d, 4 H, 2 × Ar CH_2O), 3.83 (s, 3 H, Ar OCH_3), 3.78 (s, 6 H, 2 × Ar OCH_3), 3.56–3.30 (m, 4 H, Ar CH_2C), 2.72–2.56 (t, 2 H, CH_2N); IR (KBr) 3320 (NH), 1645 (C=O) cm^{-1}.

1-(3,4,5-Trimethoxybenzyl)-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline (13). A mixture of 6.40 g (11.8 mmol) of amide **4** in 80 mL of dry acetonitrile along with 6 mL (64.4 mmol) of $POCl_3$ was refluxed under argon for 3.25 h. The mixture was allowed to cool prior to removal of solvent and excess $POCl_3$ under vacuum. The resulting residue was dissolved in 100 mL of $CHCl_3$ and washed once with 100 mL of H_2O, twice with 100 mL of saturated $NaHCO_3$, and twice with 100 mL of H_2O. The organic layer was dried over $MgSO_4$, and $CHCl_3$ was removed by use of a rotary evaporator. The residue imine oil was dissolved in 80 mL of ethanol and cooled in ice–water, and 9.00 g (238 mmol) of $NaBH_4$ was added. The mixture was allowed to warm up to room temperature, and the stirring under a $CaCl_2$ drying tube continued overnight (16 h). The solvent was removed under vacuum, 100 mL of $CHCl_3$ was added to the residue, and the resulting solution was washed twice with 100 mL of 10% NaOH and twice with 100 mL of H_2O. The $CHCl_3$ layer was dried over $MgSO_4$ and concentrated to a small volume, and HCl gas was bubbled into this remaining solution to make the HCl salt. The $CHCl_3$ and excess HCl were removed under vacuum. The residue amine salt was crystallized in $CHCl_3$/ether to give 4.74 g (72%) of a white crystalline solid: mp 205–207 °C (lit.[15] mp 199–202 °C); ^1H NMR ($CDCl_3$, free base) δ 7.50–7.29 (m, 10 H, Ar H), 6.77 (s, 1 H, Ar H), 6.69 (s, 1 H, Ar H), 6.42 (s, 2 H, Ar H), 5.13 (s, 2 H, Ar CH_2O), 5.10 (s, 2 H, Ar CH_2O), 4.11–4.06 (m, 1 H, Ar CHN), 3.84–3.32 (d, 9 H, 3 × Ar OCH_3), 3.20–2.62 (m, 7 H, NH, 3 × CH_2).

1-(3,4,5-Trimethoxybenzyl)-2-benzyl-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (7). The Bischler–Napieralski cyclization was done as in the synthesis of **13**. Thus 4.00 g (7.39 mmol) of the amide **4**, 50 mL of dry acetonitrile, and 2.0 mL (21.46 mmol) of $POCl_3$ were refluxed under argon for 3 h. Workup was done as before to obtain the imine oil. To the imine in 70 mL of benzene was added 2.4 mL (20.18 mmol) of benzyl bromide. The mixture was refluxed for 3.5 h under argon during which time a yellow solid crystallized. It was then allowed to cool to room temperature and filtered under suction, and the crystalline material obtained was washed with ether to give 4.90 g (95%) of iminium salt **6**: mp 199–202 °C (lit.[15] mp 199–202 °C).

To a mixture of 3.30 g (4.75 mmol) of the salt in 100 mL of ethanol was added 1.17 g (30.93 mmol) of $NaBH_4$ during cooling with ice–water. The mixture was then allowed to warm to room temperature, and stirring continued overnight. The ethanol was removed under vacuum, and $CHCl_3$ was added to the residue. The suspension was washed three times with water and dried over Na_2SO_4, and the HCl salt was prepared. Crystallization in methanol/ether gave 2.85 g (92%) of **7** as a white crystalline solid: mp 166–168 °C; ^1H NMR ($CDCl_3$, free base) δ 7.41–7.21 (m, 15 H, Ar H), 6.69 (s, 1 H, Ar H), 6.29 (s, 1 H, Ar H), 6.21 (s, 2 H, Ar H), 5.11 (s, 2 H, Ar CH_2O), 4.92 (s, 2 H, Ar CH_2O), 3.38 (s, 3 H, Ar OCH_3), 3.75 (s, 2 H, Ar CH_2N), 3.72 (s, 6 H, 2 × Ar OCH_3), 3.46–2.53 (m, 7 H, CH, 3 × CH_2); MS, 615 (M^+ – HCl), 434, 181, 91 (base). Anal. ($C_{40}H_{42}NO_5Cl$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8). Method A. To 1.60 g (2.45 mmol) of **7** was added 100 mL of 50:50 solution of methanol/concentrated HCl, and the mixture was refluxed for 32 h while being stirred under argon. The solvent and excess HCl were removed under vacuum. Methanol was added to the residue and then removed under vacuum, and this step was repeated. Crystallization in ethanol/ether gave 1.14 g (99%) of whiite crystalline **8**: the $FeCl_3$ test for catechols was positive; mp 160–162 °C.

An analytical sample was prepared by dissolving 390 mg (0.83 mmol) in H_2O, washing the solution three times with $CHCl_3$, and removing the water azeotropically with methanol. Crystallization with ethanol/ether gave 262 mg (67%) of white crystalline **8**: the $FeCl_3$ test for catechols was positive; mp 160–162 °C; ^1H NMR (D_2O) δ 7.45–7.10 (m, 5 H, Ar H), 6.70 (s, 1 H, Ar H), 6.11 (s, 2 H, Ar H), 6.05 (s, 1 H, Ar H), 4.22 (br s, 2 H, Ar CH_2N), 3.80–2.90 (m, 16 H, 3 × Ar OCH_3, CH, 3 × CH_2); IR (KBr) 3220 (OH) cm^{-1}; MS, 435 (M^+ – HCl), 254 (base), 181, 91. Anal. ($C_{25}H_{30}N_{14}O_5Cl$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-benzyl-6,7-diacetyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (9). To 354 mg (0.750 mmol) of catechol **8** (before $CHCl_3$ washing) in 5 mL of $CHCl_3$ was added 1.5 mL of Et_3N and 110 mg of 4-(dimethylamino)-pyridine (DMAP) and 0.20 mL (2.120 mmol) of acetic anhydride during cooling with ice–water. The mixture was allowed to warm up to room temperature and stirred overnight. The mixture was then washed twice each with H_2O, saturated $NaHCO_3$, and H_2O. It was dried over $MgSO_4$, concentrated, and purified by flash chromatography using 45% ethyl acetate in hexane as eluting solution. The HCl salt was prepared, and crystallization using $CHCl_3$/ether gave 203 mg (49%) of white crystalline diacetate **9**: mp 181–183 °C; ^1H NMR ($CDCl_3$, free base) δ 7.21 (s, 5 H, Ar H), 6.93 (s, 1 H, Ar H), 6.56 (s, 1 H, Ar H), 6.20 (s, 2 H, Ar H), 3.84 (s, 3 H, Ar OCH_3), 3.78 (s, 2 H, NCH_2Ph), 3.73 (m, 6 H, Ar OCH_3), 3.30–2.61 (m, 7 H, CH, 3 × CH_2), 2.27 (s, 3 H, CH_3C=O), 2.23 (s, 3 H, CH_3C=O); IR (KBr) 1775, 1765 (C=O) cm^{-1}; MS, 519 (M^+ – HCl), 338 (base), 296, 53, 181, 91. Anal. ($C_{30}H_{34}NO_7Cl$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8). Method B. A mixture of 54 mg (0.097 mmol) of the diacetate **9** in 3 mL of ethanol/concentrated HCl (50:50) under argon was stirred and refluxed for 2 h. The solvents were removed under vacuum.

Methanol was added to the residue was removed by rotavap and this process repeated. Crystallization in THF/ether (or ethanol/ether) gave 44 mg (96%) of catechol 8. This compound was identical with 8 prepared by method A.

1-(3,4,5-Trimethoxybenzyl)-2-(chloroacetyl)-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (14). To a mixture of 2.30 g (4.09 mmol) of amine salt 13 in 50 mL of dry acetonitrile was added 1.60 g (15.09 mmol) of Na_2CO_3 followed by dropwise addition of a solution of 1.91 g (11.17 mmol) of chloroacetic anhydride [$(ClCH_2CO)_2O$] in 20 mL of dry acetonitrile. The mixture was then stirred at room temperature overnight (20 h). The mixture was filtered and the filtrate concentrated under vacuum. The residue was redissolved in $CHCl_3$ and washed twice with 50 mL of 10% Na_2CO_3 and then H_2O. The organic layer was dried over Na_2SO_4 and concentrated to give a yellow oil, which turned solid. Purification of the solid by column chromatography using silica gel (particle size 0.063–0.2 mm) as packing and ethyl acetate/hexane (6:4, then 6.5:3.5) as eluting solution was carried out. The appropriate fractions were combined, concentrated, and crystallized with use of CH_2Cl_2/hexane to give 1.97 g (85%) of white crystalline amide: mp 124–125 °C; ^1H NMR ($CDCl_3$) δ 7.39–7.33 (m, 10 H, Ar H), 6.37 (s, 1 H, Ar H), 6.30 (s, 1 H, Ar H), 6.17 (s, 2 H, Ar H), 5.12, 5.10 (d, 4 H, Ar CH_2O), 4.10 (s, 2 H, O=CCH_2Cl), 3.83–2.56 (m, 16 H, 3 × Ar OCH_3, CH, 3 × CH_2); IR (KBr) 1640 (C=O) cm^{-1}; MS, 420, 386, 181, 91 (base). Anal. ($C_{35}H_{36}NO_5Cl$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-(β-hydroxyethyl)-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (17). A solution of 2.40 g (4.27 mmol) of amine salt 13 in $CHCl_3$ was washed three times with saturated $NaHCO_3$ solution and once with H_2O to form the amine. The $CHCl_3$ solution was dried over $MgSO_4$, concentrated, and redissolved in 80 mL of ethanol. The solution was cooled with ice–water, 100 mL of ethylene oxide was added, and stirring at this temperature was continued for 10 h. The mixture was then allowed to warm to room temperature and stirred overnight (10 h). Ethanol and excess ethylene oxide were removed under vacuum. The residue amine alcohol was redissolved in methanol and HCl gas bubbled in to make the HCl salt. The methanol and excess HCl were removed under vacuum. Crystallization using MeOH/ether gave 1.97 g (76%) of the amine alcohol salt 17: mp 98–100 °C; ^1H NMR ($CDCl_3$, free base) δ 7.52–7.29 (m, 10 H, Ar H), 6.68 (s, 1 H, Ar H), 6.39 (s, 1 H, Ar H), 6.32 (s, 2 H, Ar H), 5.12 (s, 2 H, Ar CH_2O), 4.98 (s, 2 H, Ar CH_2O), 3.82 (s, 9 H, 3 × Ar OCH_3), 3.75–2.45 (m, 7 H, CH, 3 × CH_2); IR (KBr) 3640 (OH), 3230 (OH) cm^{-1}; MS, 569 (M^+ – HCl), 388, 181, 91 (base). Anal. ($C_{35}H_{40}NO_6Cl·H_2O$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-(β-hydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (18). Hydrogenation of 970 mg (1.60 mmol) of amine alcohol salt 17 in 50 mL of dry ethanol with 86 mg of 10% Pd/C as catalyst was carried out at 45 psi for 16 h. The catalyst was filtered off and the filtrate concentrated to a small volume and passed over Celite. The remaining ethanol was removed under vacuum. Attempts to crystallize the oil obtained in several solvent systems were not successful. The oil was then placed under high vacuum to give 617 mg (91%) of a yellow fluffy solid, which gave a positive $FeCl_3$ test for catechols.

An analytical sample was prepared by dissolving 226 mg of the catechol above in water, washing the water solution three times with $CHCl_3$, and removing the water azeotropically with methanol using rotavap. The oil residue was placed under vacuum to give 0.197 mg (87%) of a yellow-white solid: mp 100–104 °C; ^1H NMR (D_2O) δ 6.64 (s, 1 H, Ar H), 6.27 (s, 2 H, Ar H), 5.86 (s, 1 H, Ar H), 3.86–3.74 (t, 2 H, CH_2O), 3.62 (s, 3 H, Ar OCH_3), 3.61 (s, 6 H, 2 × Ar OCH_3), 3.50–2.81 (m, 9 H, CH, 4 × CH_2); IR (KBr) 3200 (br, OH) cm^{-1}; MS, 208 (base), 181. Anal. ($C_{21}H_{28}NO_6Cl·H_2O$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-(β-hydroxyethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Oxalate (18a). A solution of 1.40 g (2.31 mmol) of amine salt 17 in CH_2Cl_2 was washed three times with a saturated $NaHCO_3$ solution and twice with H_2O and dried over $MgSO_4$. To this amine in methanol was added 280 mg (2.21 mmol) of oxalic acid in methanol at room temperature. Addition of ether gave 958 mg (66%) of a white crystalline oxalate salt: mp 99–101 °C; MS, 569 (M^+ – oxalate), 388, 181, 91, base.

A mixture of 418 mg (0.664 mmol) of the oxalate salt in 30 mL of ethanol and 109 mg of 10% Pd/C was hydrogenated at 40 psi for 10 h. Filtration of the mixture was followed by removal of solvent under vacuum. The residue was redissolved in a small amount of methanol, filtered over Celite, and crystallized in MeOH/ether to give 198 mg (68%) of the oxalate catechol: the $FeCl_3$ test for catechols was positive; mp 217–218 °C. Anal. ($C_{21}H_{27}NO_6·^1/_2$oxalate$·^1/_4H_2O$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-(β-chloroethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride 6,7-Cyclic Sulfite (19). During cooling of a solution of 332 mg (0.780 mmol) of amine alcohol 18 in 15 mL of dry tetrahydrofuran under argon with ice–water, 0.3 mL (4.11 mmol) of thionyl chloride was added to the mixture. Stirring with cooling was continued for 2 h, after which the mixture was allowed to warm up to room temperature and stirred overnight (18 h). A white compound crystallized out of the solution. It was filtered under suction and washed with ether to give 334 mg (87%) of 19: mp 184–186 °C; ^1H NMR (D_2O) δ 6.67 (s, 1 H, Ar H), 6.33 (s, 2 H, Ar H), 5.95 (s, 1 H, Ar H), 3.90–2.85 (m, 20 H, 3 × Ar OCH_3, CH, 5 × CH_2); MS, 418, 72 (base), 181. Anal. ($C_{21}H_{26}NO_6Cl_2S$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-(β-chloroethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (16). A solution of 200 mg (0.408 mmol) of 19 in 10 mL of methanol/water (50:50) was stirred under argon at room temperature for 4 days. The methanol was removed under vacuum and the water removed by freeze-drying to give 180 mg (99%) of 16: mp 105–110 °C; ^1H NMR (D_2O) δ 6.65 (s, 1 H, Ar H), 6.35–6.30 (d, 2 H, Ar H), 5.91 (s, 1 H, Ar H), 3.87–2.86 (m, 20 H, 3 × Ar OCH_3, CH, 5 × CH_2); IR (KBr) 3370, 3190, (br, OH) cm^{-1}; MS, 228, 226 (base), 181. Anal. ($C_{21}H_{27}NO_5Cl_2·^3/_4H_2O$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-methyl-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (11). The amine salt 11 was prepared from amide 4 in a similar manner to that for amine salt 7. A mixture of 4.00 g (7.39 mmol) of amide 4, 100 mL of acetonitrile, and 2.20 mL (23.60 mmol) of $POCl_3$ was refluxed for 3.25 h and worked up as before. Methylation of the imine also was done in a manner similar to the benzylation. Thus to the imine was added 50 mL of toluene and 1.5 mL (24.09 mmol) of methyl iodide, and the mixture was heated at 40 °C for 10 h. A yellow solid crystallized out of the solution during the reaction. The solution was allowed to cool to room temperature, some ether added, and the mixture cooled with ice–water. The yellow crystals were filtered and washed with ether to give 3.80 g (73%) of iminium salt 10: mp 192–194 °C.

Reduction of the iminium salt also was done as before. To a suspension of 2.60 g (3.71 mmol) of iminium salt 10 in 70 mL of ethanol was added 2.60 g (68.73 mmol) of $NaBH_4$ and the mixture stirred for 8 h. The color of the mixture changed from yellow to colorless during the reaction. Ethanol was removed by rotavap and the residue redissolved in $CHCl_3$/10% NaOH. The layers were separated, and the organic layer was washed once with 10% NaOH and twice with H_2O and dried over $MgSO_4$. The HCl salt of the amine was made but could not be crystallized. It was then put on a pump to give 1.97 g (92%) of a white fluffy solid: mp 67–72 °C; ^1H NMR ($CDCl_3$, free base) δ 7.46–7.32 (m, 10 H, Ar H), 6.67 (s, 1 H, Ar H), 6.29 (s, 2 H, Ar H), 6.24 (s, 1 H, Ar H), 5.10 (s, 2 H, Ar CH_2O), 4.87, 4.86 (d, 2 H, Ar CH_2O), 3.82 (s, 3 H, Ar OCH_3), 3.76 (s, 6 H, Ar OCH_3), 3.70–2.58 (m, 7 H, CH, 3 × CH_2), 2.53 (s, 3 H, NCH_3); MS, 539 (M^+ – HCl), 358 (base), 181, 91. Anal. ($C_{34}H_{38}NO_5Cl$) C, H, N.

1-(3,4,5-Trimethoxybenzyl)-2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (12). Method A. Hydrogenation of 115 mg (0.200 mmol) of amine salt 11 in 20 mL of EtOH using 18 mg of 10% Pd/C as catalyst was carried out at 40 psi for 16 h. Filtration was followed by removing the ethanol and crystallizing in MeOH/ether to give 48 mg (61%) of catechol 12 as a white–light brown crystalline material: the $FeCl_3$ test for catechols was positive; mp 219–221 °C.

Method B. A mixture of 333 mg (0.578 mmol) of amine salt 11, 10 mL of MeOH, and 10 mL of concentrated HCl was refluxed for 30 h. Methanol and HCl solution were then removed under vacuum. The residue was redissolved in MeOH and the MeOH removed under vacuum. This procedure was repeated twice and gave a yellow solid. Crystallization in MeOH/ether gave 154 mg (67%) of catechol 12 as a white crystalline material: the $FeCl_3$

test for catechols was positive; mp 222–224 °C; ^{1}H NMR (D_2O) δ 6.66 (s, 1 H, Ar H), 6.27 (s, 2 H, Ar H), 5.96 (s, 1 H, Ar H), 3.64 (s, 3 H, Ar OCH_3), 3.62 (s, 6 H, Ar OCH_3), 3.50–2.90 (m, 7 H, CH, 3 × CH_2), 2.82 (s, 3 H, NCH_3); IR (KBr) 3455 (OH) cm^{-1}; MS, 359 (M^+ – HCl), 181, 178 (base). Anal. ($C_{20}H_{26}NO_5Cl$) C, H, N.

Biological Studies. β-Adrenergic Studies. Male albino Hartley guinea pigs (400–600 g) were employed in all experiments. The isolation and procedures for testing of each compound with isolated guinea pig atria and trachea were identical with those described by Miller et al.[34] Each drug concentration was added only after the effects of the previous concentration reached a maximum and remained constant. The final maximum concentration was considered to be that response at which further addition of a higher concentration of the testing compound did not increase the effect. Responses for agonists were expressed as pD_2 values (–log ED_{50}) and were calculated directly from graphical plots of percent maximal response vs. log molar drug concentration. All TMQ analogues were prepared as 0.01 M solutions in 0.9% saline containing 0.05% metabisulfite.

Platelet Antiaggregatory Activities. Materials. Compounds and sources were as follows: [^{14}C]serotonin (58 mCi/mmol) and Formula 963 (New England Nuclear, Boston, MA); albumin, apyrase, and Tris base (Sigma Chemical Co., St. Louis, MO); 15(*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5(*E*),13(*Z*)-dienoic acid, U46619 (Upjohn Diagnostics, Inc., Kalamazoo, MI); and trimetoquinol isomers (provided by Dr. Yoshio Iwasawa of Tanabe Seiyaku Co., Ltd., Saitama, Japan).

Methods. Isolation and preparation of washed human platelets were done essentially as reported previously.[13] Blood was taken by venipuncture from normal healthy donors who had been free of aspirin-containing medication for at least 14 days. Whole venous blood was mixed with acid-citrate dextrose (ACD) anticoagulant (6:1, v/v) and centrifuged at 120*g* for 15 min at room temperature to obtain platelet-rich plasma (PRP). PRP was centrifuged at 1100*g* for 3 min, and the resulting platelet pellet was resuspended in 50 mM Tris·HCl (pH 7.5) buffer solution containing 20 mM EDTA. Apyrase (EC 3.6.1.5) (34 μg/mL) also was added to this suspension to prevent accumulation of ADP. The suspension was centrifuged at 1100*g* for 3 min for first wash. The washing procedure was repeated three times, and the final platelet pellet was resuspended in a modified Tyrode's solution (pH 7.4) containing calcium. Platelet counts were adjusted to 3 × 10^8 platelets/mL to perform functional studies.

Measurement of Platelet Aggregation. Platelet aggregation studies were performed according to the turbidometric method of Born, as modified by Mustad et al., using a Payton aggregometer.[35,36] Incubation of 0.45 mL of washed human platelets with 0.05 mL of vehicle or drug (total volume = 0.5 mL) was carried out for 3 min at 37 °C prior to the initiation of aggregation with U46619. This time period also served as the incubation interval for the TMQ isomers and analogues. In all experiments, the minimum concentration of U46619 (0.8–2.0 μM) that caused maximal irreversible aggregation within each preparation was used. Aggregation was monitored for at least 2 min after the addition of U46619, and data were expressed as a percentage inhibition of the maximal light transmittance to U46619 in the presence of varying drug concentrations.

Measurement of [^{14}C]Serotonin Secretion. Secretion of serotonin from dense granules was measured by monitoring the release of radioactivity[37] from the same samples used for measurement of platelet aggregation. Release of serotonin from platelets was measured by centrifugation of samples at 10000*g* for 30 s in a microfuge and determining the radioactivity present in an aliquot (100 μL) of the supernatant. Total radioactivity was measured after lysis of platelets with Protosol solubilizer (1 mL). Liquid scintillation spectrometry using a Beckman liquid scintillation counter (Model LS 6800, Palo Alto, CA) and an emulsion-type scintillation mixture (Formula 963) was used to determine the amount of the ^{14}C in supernatants of lysed or nonlysed samples.

Secretion data were calculated as the net increase of serotonin released into the supernatant by U46619 and expressed as a percentage of the total radioactivity in platelets. The effects of compounds on serotonin release were expressed as the percentage inhibition of the maximum release by U46619 and plotted against log molar concentrations of each agent.

Determination of Inhibitory Concentration–50 (IC_{50}) Values. IC_{50} values for each drug were estimated from graphical plots of % inhibition vs. log molar concentration of each drug and were defined as the concentrations required to produce 50% inhibition of the maximal aggregatory or secretory response to U46619. Data were expressed as the mean ± standard error of the mean (SEM) of results obtained from three of more preparations.

Acknowledgment. We thank NIH for financial support (Grant No. HL22533). Assistance from Hebattala Amin in the syntheses of compounds 12 and 14 is acknowledged.

Registry No. (+)-1, 18559-60-9; (–)-1, 18559-59-6; (±)-1, 18559-63-2; 2, 55536-65-7; 2·HCl, 1699-56-5; 3, 951-82-6; 4, 37481-62-2; 5, 55536-67-9; 6, 64125-88-8; 7, 102920-94-5; 7 (free base), 102920-93-4; 8, 102920-95-6; 9, 102920-96-7; 9 (free base), 102920-97-8; 10, 102921-06-2; 11, 102921-05-1; 12, 102921-07-3; 13, 102921-08-4; 13·HCl, 37602-12-3; 14, 102940-16-9; 14 (free base), 102940-17-0; 16, 102921-04-0; 17, 102920-99-0; 17 (free base), 102920-98-9; 17 (oxalate), 102921-01-7; 18, 102921-00-6; 18 (free base), 102921-02-8; 18a, 102921-03-9; 19, 102940-18-1.

(34) Miller, D. D.; Osei-Gyimah, P.; Bardin, J.; Feller, D. R. *J. Med. Chem.* **1975**, *18*, 454.
(35) Born, G. V. R. *Nature (London)* **1962**, *194*, 927.
(36) Mustard, J. F.; Perry, D. W.; Ardlie, N. G.; Packham, M. A. *Br. J. Haematol.* **1972**, *22*, 193.
(37) Costa, J. L.; Murphy, D. L. *Nature (London)* **1975**, *255*, 407.